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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Lipid Membrance Structures for Oral Administration

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(30) (JP) 132385/1991 1991/03/25

(57) 15 Claims

Abstract of the Disclosure

Lipid membrane structures for oral administration contain phosphatidylserine, a mannan derivative or a mannose derivative as ingredient of the membrane. In particular, the lipid mambrane structures contain a drag therein, the drug being surrounded with the membrane. It is in the form of liposomes, emulsions or micelles and capable of efficiently delivering a drug contained therein to Peyer's patches in the small intestines. Therefore, the lipid membrane structures of the present invention are excellent as oral dosage lipid membrane structures.

The embodiments of the invention, in which an exclusive property or privilege is claimed are defined as follows:

1. Lipid membrane structures for oral administration containing phosphatidylserine as an ingredient of the membrane.

2. Lipid membrane structures of claim 1 wherein the membrane comprises phosphatidylserine at least 1 mole % based on the total components of the membrane.

3. Lipid membrane structures of claim 2 wherein the membrane comprises phosphatidylserine in an amount of 5 to 50 mole % based on the total components of the membrane.

4. Lipid membrane structures of claim 3 wherein a drug is contained therein and the drug is surrounded with the membrane which comprises phosphatidylserine and a component selected from the group consisting of an amphiphilic substance and a micelle-forming surfactant.

5. Lipid membrane structures of claim 4 wherein the component is lecithin or sphingomyelin.

6. Lipid membrane structures of claim 5 wherein the membrane comprises a stabilizer.

7. Lipid membrane structures of claim 1 wherein a mannose derivative or a mannan derivative is incorporated in the membrane thereof.

8. Lipid membrane structures of claim 4 wherein a mannose derivative or a mannan derivative is incorporated in the membrane thereof.

9. Lipid membrane structures of claim 4 wherein a mannan derivative is incorporated in the membrane thereof.

10. Lipid membrane structures of claim 7 wherein the mannose derivative is selected from the group consisting of phosphatidylethanolamine derivatives having a mannose residue, cholesterol derivatives having a mannose residue, dimannosyl diglyceride, mannobiose fatty acid esters and amides and phosphatidyl mannose.

11. Lipid membrane structures of claim 7 wherein the mannan derivative is selected from the group consisting of mannan partially substituted with a fatty acid and mannan partially substituted with cholesterol.

12. Lipid membrane structures for oral administration wherein a mannose derivative or a mannan derivative is incorporated in the membrane thereof.

13. A method for delivering a drug to Peyer's patches in a digestive tract comprising orally administering lipid membrane structures in which the drug is contained therein and the drug is surrounded with a

membrane which comprises phosphatidylserine and a component selected from the group consisting of an amphiphilic substance and a micelle-forming surfactant.

14. A method of claim 13 wherein the component is lecithin or sphingomyelin.

15. A method of claim 13 wherein the lipid membrane structures contains a mannose derivative or a mannan derivative in the membrane thereof.

Specification

Title of the Invention

Lipid Membrane Structures for Oral Administration

Background of the Invention

The present invention relates to lipid membrane structures capable of efficiently reaching Peyer's patches in a digestive tract after oral administration. The lipid membrane structures prepared by the present invention are capable of efficiently delivering a drug contained therein to Peyer's patches in a digestive tract.

Recently intensive investigations are made on a drug delivery system (DDS) for the purpose of improving the effect, safety and usage of a drug. DDS is classified to a contral-release type (the first generation DDS) and a targeting contral type (the second generation DDS) in the scientific field.

As for the drugs for oral administration, the investigations have been made heretofore on mainly DDS of the former type (control-release type) but the DDS of the latter type (targeting type) has been scarcely investigated. The Peyer's patch in a digestive tract is a protrusion in a mucous membrane of the small intestine and it is an aggregate of limph follicles. It is also a tissue recently attracting attention in this technical field and investigations are made for the purpose of elucidating the immunological roles thereof. Investigations are made on the mechanism of uptake of latex beads through Peyer's patches [Clinical Experimental Immunology, Vol. 76, p.

144 (1989)] and uptake of a drug enclosed in a biodegradable polymer through Peyer's patches (European Patent Application No. 87309286.0). However, according to these conventional techniques, it is impossible for a drug to be selectively taken up through Peyer's patches in a digestive tract after oral administration.

Investigations were made also on the use of liposomes for an oral drug. Namely, investigations were made on encapsulating, in liposomes, a high-molecular drug such as insulin [Biochimica et Biophysica Acta, Vol. 716, p. 188 (1982)], blood coagulation factor VIII [Lancet, i, p. 70 (1980)] or heparin [Chemical and Pharmaceutical Bulletin, Vol. 30, p. 2245 (1982)]; a hardly water-soluble drug such as vitamin K_1 [Journal of Pharmacy and Pharmacology, Vol. 36, p. 527 (1984)] or griseofulvin [Journal of Pharmaceutical Sciences, Vol. 73, p. 757, (1984)]; or a Streptococcus cell wall antigen [Immunology, vol. 54, P. 189(1985)] (for the purpose of production of IgA antibody). However, an idea of modifying the liposomal membrane with an additive so as to impart a selectivity toward Peyer's patches in the digestive tract has never been reported.

Further, as for the liposomes used as an injection, it was reported that when the membrane thereof was modified with phosphatidylserine [Cancer Research, Vol. 40, p. 4460 (1980)], alveolar macrophages were activated to improve the antitumor effect after intravenous injection and that when it was modified with a mannose derivative [Biochimica et Biophysica Acta, Vol. 632, p. 562 (1980)] or a mannan derivative ["Byotai Seiri", Vol. 6, p. 771 (1985)], the liposomes were distributed to the liver and the lung.

Thus the efficient delivery of a drug to Peyer's patches in a digestive tract after oral administration has not yet been proposed in the prior art.

Summary of the Invention

A primary object of the present invention is to provide lipid membrane structures capable of efficiently delivering a drug therein to Peyer's patches in a digestive tract after oral administration.

This and other objects of the present invention will be apparent from the following description and examples.

The present invention has been completed on the basis of a finding that the above-described objects can be attained by using at least one of phosphatidylserines, mannose derivatives and mannan derivatives as a component of a membrane of lipid membrane structures.

Brief Description of the Drawings

Fig. 1 shows the results of the distribution of PS-liposomes of the present invention and SA-liposomes to Peyer's patches.

Fig. 2 shows the effect of the amount of PS content of PS-liposomes of the present invention on the distribution to Peyer's patches.

Fig. 3 shows changes of the distribution of PS-liposomes of the present invention to Peyer's patches with time after administration of the test dispersion.

Fig. 4 shows the specificity of PS-liposomes of the present invention for the portions of the intestinal tract in the distribution

to Peyer's patches and non-Peyer's patches.

Fig. 5 shows an improvement of the distribution of PS-liposomes of the present invention to Peyer's patches by modifying it with mannan derivatives.

Fig. 6 shows an improvement of the distribution of PS-liposomes of the present invention to Peyer's patches by modifying with mannan derivatives.

The abbreviations in the above brief description are referred in Example hereinafter.

Description of the Preferred Embodiments

The lipid membrane structures of the present invention indicate lipid particles having such membrane structures wherein polar head groups of amphiphilic lipids are arranged at the interface toward an aqueous phase. Examples of the lipid membrane structures include liposomes, emulsions and water-soluble micelles. The lipid membrane structures are characterized in that phosphatidylserines and/or mannose derivatives and/or mannan derivatives are incorporated therein as a component of the membrane so that the lipid membrane structures can be efficiently distributed to Peyer's patches in a digestive tract after oral administration.

The phosphatidylserines used in the present invention include natural soybean or egg-yolk phosphatidylserine, hydrogenated phosphatidylserine obtained by hydrogenation of the natural soybean or egg-yolk phosphatidylserine and semi-synthetic dimyristoyl

phosphatidylserine, dipalmitoyl phosphatidylserine and distearoyl phosphatidylserine. Among them, preferred are hydrogenated phosphatidylserine, dipalmitoyl phosphatidylserine and distearoyl phosphatidylserine.

The mannose derivatives used in the present invention include phosphatidylethanolamine derivatives having a mannose residue [Biochimica et Biophysica Acta, Vol. 632, p. 562 (1980)], cholesterol derivatives having a mannose residue [Proceeding of National Academic Scienses U.S.A. Vol. 77, p. 4430 (1980)], dimannosyl diglyceride [Biochemical and Biophysical Research Communications, Vol. 110, p. 140 (1983)], mannobiose fatty acid esters and amides (preferably, fatty acid residue having 12 to 30 carbon atoms)[Japanese Patent Unexamined Published Application (hereinafter referredto as "J.P. KOKAI") No. Hei 1-104088], phosphatidyl mannose, etc.

The mannan derivatives in the present invention include, for example, mannan (a polysaccharide) partially substituted with a fatty acid or cholesterol (preferably, fatty acid residue having 12 to 30 carbon atoms)["Byotai Seiri", Vol. 6, p. 771 (1985)].

These additives used as a component of the membrane are used either singly or in the form of a mixture of them and they usually can be incorporated into the membrane of the lipid membrane structures mainly comprising lecithin (i.e., phosphatidylcholine) or the like to modify the membrane of the lipid membrane structures. Alternatively, they can singly form the lipid membrane structures. Namely, the phosphatidylserines per se can form liposomes or emulsions, and mannose derivatives such as mannobiose fatty acid esters and amides

thereof can singly form emulsions or micelles. Therefore, the upper limit of the relative amount of the phosphatidylserines, mannose derivatives or mannan derivatives used as the component of the lipid membrane structures is not limited at all in the present invention. The lower limit of the molar ratio is 1 % based on the total membrane components of the lipid membrane structures. Preferably, the amount of them used is 5 to 50 %.

The drugs which can be contained in the lipid membrane structures of the present invention vary depending on the kind of the lipid membrane structures. For example, drugs which can be encapsulated in the liposomes are not particularly limited and they include high-molecular compounds such as insulin, hormones and polysaccharides; water-soluble drugs such as immunopotentiators and immunosuppressants; and fat-soluble drugs. The drugs which can be contained in the emulsions include fat-soluble drugs and those which can be contained in the micelles include hardly water-soluble drugs. They can be kept in the lipid membrane structures prepared by ordinary processes which are described below.

The description will be made on processes for preparing the lipid membrane structures of the present invention containing phosphatidylserines and/or mannonose derivatives and/or mannan derivatives added thereto.

a) Process for preparing liposomes

An aqueous dispersion of liposomes is prepared from membrane-forming components such as lecithin or sphingomyelin and additive(s), i.e. phosphatidylserines and/or mannose derivatives and/or mannan

derivatives by a known process [Annual Review of Biophysics and Bioengineering, Vol. 9, p. 467 (1980)]. The thus-prepared liposomes may contain stabilizers such as sterols, e.g. cholesterol, charged lipids such as dialkyl phosphate, diacylphosphatidic acid or stearylamine, or antioxidants such as α -tocopherol.

b) Process for preparing emulsions

An aqueous dispersion of emulsions can be prepared from amphiphilic substances such as lecithins or polyoxyethylene sorbitan fatty acid esters (Tween's), additive(s), i.e. phosphatidyl serines and/or mannose derivatives and/or mannan derivatives, and oil such as soybean oil by a known emulsion-preparation process.

c) Process for preparing micelles

An aqueous dispersion of micelles can be prepared from micelle-forming surfactants (to be added to an aqueous solvent at a concentration not lower than a critical concentration of micelle formation) such as polyoxyethylene sorbitan fatty acid esters (Tween's), sodium salt of fatty acid or polyoxyethylene hydrogenated castor oil and additive(s), i.e. posphatidylserines and/or mannose derivatives and/or mannan derivatives by a known micelle-preparation process.

The lipid membrane structures of the present invention are capable of efficiently delivering a drug contained therein to Peyer's patches after oral administration. The Peyer's patch is an aggregate of lymph follicles in the mucus membrane of the small intestine and it is one of tissues concerned with absorption of drugs. In particular, it is a quite important tissue for absorption of

particularly high-molecular drugs or drugs relating to control of immunological activity.

Therefore, in case where a drug which could not be absorbed well when it was given by oral administration is encapsulated in the lipid membrane structures of the present invention, its absorption can be improved.

Therefore, the lipid membrane structures of the present invention are very unigue and excellent as oral lipid membrane structures for drugs which have been hardly able to be administrated orally by conventional techniques.

The following Examples and Test Examples will further illustrate the present invention, which by no means limit the invention. The basic method for preparation of the liposomes and experiments of the distribution of the liposomes to the Peyer's patches are summarized below.

Preparation of liposomes (Examples 1 to 4 and Control Examples 3 and 4)

Lipids in a molar ratio given below were placed in a short-neck flask in a total amount of 50 μ mol. They were thoroughly dissolved in a mixture of chloroform and methanol and then the organic solvents were distilled off in an evaporator under reduced pressure. The organic solvents were completely removed under reduced pressure in a desiccator to dry the lipid mixture. Then 2 ml of tris-hydrochloric acid-buffered physiological saline (pH 7.4) containing 10 mg/ml of 6-carboxyfluorescein (6-CF) or 10 mg/ml of phenol red which was an inner aqueous phase marker was added thereto

and they were stirred and shaken in a vortex mixer at 50℃ . Then the dispersion was passed through a polycarbonate membrane filter having a pore diameter of 0.4 μ m at 50℃ to obtain a liposomal dispersion having a particle diameter of not larger than 0.4μ m.

The inner aqueous phase marker which remained not encapsulated in the liposomes was removed by applying the thus-obtained liposomal dispersion through a column of Sephadex G-100 (3 cm diameter x 45 cm; elution buffer: tris-hydrochloric acid-buffered physiological saline having pH of 7.4). The liposomal fractions containing the inner aqueous phase marker were finally diluted with the tris-hydrochloric acid-buffered physiological saline (pH 7.4) to 6-CF concentration of 4.0 μ g/ml or phenol red concentration of 400 μ g/ml (final total lipid concentration: about 1 mM = 1 μ mol/ml).

The liposomes modified with mannan derivative in Example 4 were prepared by first preparing liposomes having PC/PS/CHOL molar ratio of 7/3/2 in the same manner as that of Example 2 and then reacting the liposomes with a mannan-cholesterol derivative [the amount of the mannan-cholesterol derivative was 53.1 μ g (in terms of mannan) per μ mol of the phospholipid in the liposome] at 4℃ for 2 h.

Control Ex. 3 : PC/PS/CHOL molar ratio = 7/0/2

Example 1 : PC/PS/CHOL molar ratio = 7/1/2

Example 2 : PC/PS/CHOL molar ratio = 7/3/2

Example 3 : PC/PS/CHOL molar ratio = 7/5/2

Example 4 : PC/PS/CHOL/Man-CHOL

Control Ex. 4 : PC/SA/CHOL molar ratio = 7/3/2

[Symbols]

PC : egg yolk phosphatidylcholine

PS : hydrogenated soybean phosphatidylserine

CHOL : cholesterol

Man-CHOL : mannan-cholesterol derivative [N-(2-N-cholesterylcarboxyaminoethyl)carbamylmannan, trade name: Chol-AECM-Mannan (a product of Wako Jun'yaku Co.)]

SA : stearylamine

Experiment on distribution of liposomes to Peyer's patches (Test Examples 1 to 6)

The distribution of the liposomes to the Peyer's patches was investigated by the in situ loop method. In the test, male Wistar rats weighing around 250 g were made to fast for 18 hours. After the celiotomy conducted under anesthesia with urethane, the small intestine was ligatured to divide it into four parts each having a length of about 15 cm to form loops. Then 1.0 ml of a test solution or dispersion (containing 4.0 μg of 6-CF, 400 μg of phenol red or about 1μ mol of total lipids) was injected in each loop. After a given period of time, the intestinal tract was taken out and washed with a physiological saline solution. Twenty mm^2 of the tissue of the Peyer's patches and a tissue around the Peyer's patch (non-Peyer's patches) were cut and taken and the inner aqueous phase marker delivered to the both tissues was extracted with isoamyl alcohol and determined.

Test Example 1

The test solution and dispersions of Control Example 1 (4.0 μ g/ml of free 6-CF), Control Example 3 (PC-liposomes) and Example 2 (PS-liposomes) were used in the test of delivery of the markers to the Peyer's patches. The results obtained two hours after injection are shown in Fig. 1 (left) and Table 1. It is evident that the delivery of 6-CF encapsulated in the PC-liposomes or PS-liposomes to the Peyer's patches and non-Peyer's patches was improved as compared with that of free 6-CF. It is also evident that the distribution of PS-liposome to the Peyer's patches was significantly improved as compared with that to the non-Peyer's patches.

Table 1

Dispersion / Tissue	Control Example 1 Free 6-CF	Control Example 3 PC-liposomes	Example 2 PS-liposomes	Significant difference (level of significance
Peyer's patch tissue (number of times of repetition	3.53 ± 0.34 (n=21)	13.67 ± 1.67 (n=13)	50.81 ± 3.52 (n=19)	Significant difference was found in Example 2 (1%)
Significance difference (level of significance)	Yes (1%)		Yes (1%)	
Non-Peyer's patch tissue (number of times of repetition)	3.35 ± 0.41 (n=20)	12.46 ± 1.62 (n=16)	30.71 ± 2.66 (n=17)	

Unit: ng/mm² × 10^{-2}, mean ± standard error

Test Example 2

The distribution test to the Peyer's patches was conducted with the test solution and dispersion of Control Example 2 (400μ g/ml of free phenol red) and Control Example 4 (SA-liposome). The results obtained two hours after injection are shown in Fig. 1 (right) and Table 2. The distribution to the Peyer's patches and non-Peyer's patches was similar to that observed when free phenol red was given, and no significant difference was recognized.

Table 2

Dispersion / Tissue	Control Example 2 Free phenal red	Control Example 4 SA-liposomes
Peyer's patch tissue (number of times of repetition	4.35 ± 0.55 (n=2)	5.27 ± 0.73 (n=6)
Non-Peyer's patch tissue (number of times of repetition)	4.60 ± 0.44 (n=3)	3.77 ± 0.13 (n=3)

Unit: $ng/mm^2 \times 10^{-3}$, mean ± standard error

Test Example 3

The influence of the amount of PS on the delivery of 6-CF to the Peyer's patches was examined. In the experiment of the distribution to the Peyer's patches, PC-liposomes of Control Example 3 and PS-liposomes of Examples 1, 2 and 3 were used as the test dispersions. The results obtained two hours after injection are shown in Fig. 2 and Table 3. As compared with PC-liposomes, PS-liposomes exhibited an improved effect of delivering the enclosed 6-CF to the Peyer's patches as the PS content was increased. No significant difference was observed in the three PS-liposomes having different PS contents (Examples 1, 2 and 3).

Table 3

Dispersion / Tissue	Control Example 3 PC/PS/CHOL = 7/0/2	Example 1 PC/PS/CHOL = 7/1/2	Example 2 PC/PS/CHOL = 7/3/2	Example 3 PC/PS/CHOL = 7/5/2
Peyer's patch tissue (number of times of repetition	13.67 ± 1.67 (n=13)	31.31 ± 4.39 (n=10)	50.81 ± 3.52 (n=19)	44.22 ± 9.35 (n=6)
Significance difference (level of significance)	Yes (5%)		Yes (1%)	Yes (1%)
Non-Peyer's patch tissue (number of times of repetition)	12.46 ± 1.62 (n=16)	19.76 ± 2.72 (n=9)	30.71 ± 2.65 (n=17)	17.60 ± 2.83 (n=6)

Unit: ng/mm² × 10^{-2}, mean ± standard error

Test Example 4

The effect of incubation time on the distribution of PS-liposomes to the Peyer's patches and non-Peyer's patches was examined. In the experiment of the distribution to the Peyer's patches, the test dispersion of Example 2 (PS-liposome) was used. Changes with time after injection of the test dispersion are shown in Fig. 3 and Table 4. It was found that the distribution to the Peyer's patches was accelerated with the incubation time and reached the saturation after about two hours. The distribution to the non-Peyer's patches was not so remarkable and was substantially constant.

Table 4

Incubation time	30 min	1 h	2 h	3 h
Peyer's patch tissue (number of times of repetition	14.85 ± 0.95 (n=13)	21.81 ± 1.91 (n=10)	54.60 ± 5.07 (n=8)	48.61 ± 4.90 (n=11)
Non-Peyer's patch tissue (number of times of repetition)	13.84 ± 1.19 (n=11)	11.51 ± 1.35 (n=10)	12.60 ± 2.07 (n=6)	6.29 ± 0.99 (n=5)
*Significant difference (level of significance)	No	Yes (1%)	Yes (1%)	Yes (1%)

Unit: $ng/mm^2 \times 10^{-2}$, mean ± standard error

* Significant difference between movement to the Peyer's patch tissue and distribution to the non-Payer's patch tissue

Test Example 5

Specificity of the test dispersion of Example 2 (PS-liposomes) for the position of the intestinal tract to which the PS-liposomes were distributed was examined in the distribution test to the Peyer's patches and non-Peyer's patches. The results are shown in Fig. 4. No particular difference in the position was found.

Test Example 6

The test dispersions of Example 2 (PS-liposomes) and Example 4 (PS-liposomes modified with mannan derivative) were used in the test of distribution to the Peyer's patches. The results are shown in Fig. 5 and Table 5 (left). It is understood that when the membranes of PS liposomes were further modified with the mannan derivative, the amount thereof distribution to the Peyer's patches was significantly increased and that distribution to the non-Peyer's patches was significantly decreased.

The results obtained in Control Example 5 wherein the PS-liposomes modified with mannan derivative was injected together with an excess amount of the mannan derivative(100 parts per part of the mannan derivative added to the liposomes) and Control Example 6 wherein PS-liposomes modified with the mannan derivative was injected together with empty PS-liposomes free from the inner aqueous phase marker [the membrane composition was the same as that of Example 2 but 100 μ mol (100 times as much as that used in Example 2) of the lipids were used] are shown in Fig. 6 (right) and Table 5 (right). It was proved that the liposomes of the present invention has properties of selectively distribution to the Peyer's patches from

the facts that the distribution of the PS-liposomes modified with mannan derivative to the Peyer's patches was significantly inhibited by the use of the above-described combination but the distribution to the non-Peyer's patches was not particularly influenced.

Table 5

Dispersion / Tissue	Example 2: PS-Liposomes	Example 4: mannan modified PS-Liposomes	Control Example 5: Example 4 + excess amount of mannan	Control Example 6: Example 4 + empty PS-Liposomes
Peyer's patch tissue (number of times of repetition)	50.81 ± 3.52 (n=19)	73.09 ± 7.52 (n=20)	19.97 ± 1.72 (n=57)	7.99 ± 0.98 (n=35)
Significance difference (level of significance)	Yes (5%)		Yes (1%)	Yes (1%)
Non-Peyer's patch tissue (number of times of repetition)	30.71 ± 2.65 (n=17)	7.84 ± 0.98 (n=24)	13.65 ± 1.84 (n=27)	8.03 ± 1.00 (n=23)
Significance difference (level of significance)	Yes (1%)		No	No

Unit: ng/mm² × 10⁻³, mean ± standard error

Fig.1 EFFECT OF LIPID COMPOSITION ON THE UPTAKE OF LIPOSOMES

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Fig.2 EFFECT OF PS CONTENT ON THE UPTAKE OF LIPOSOMES

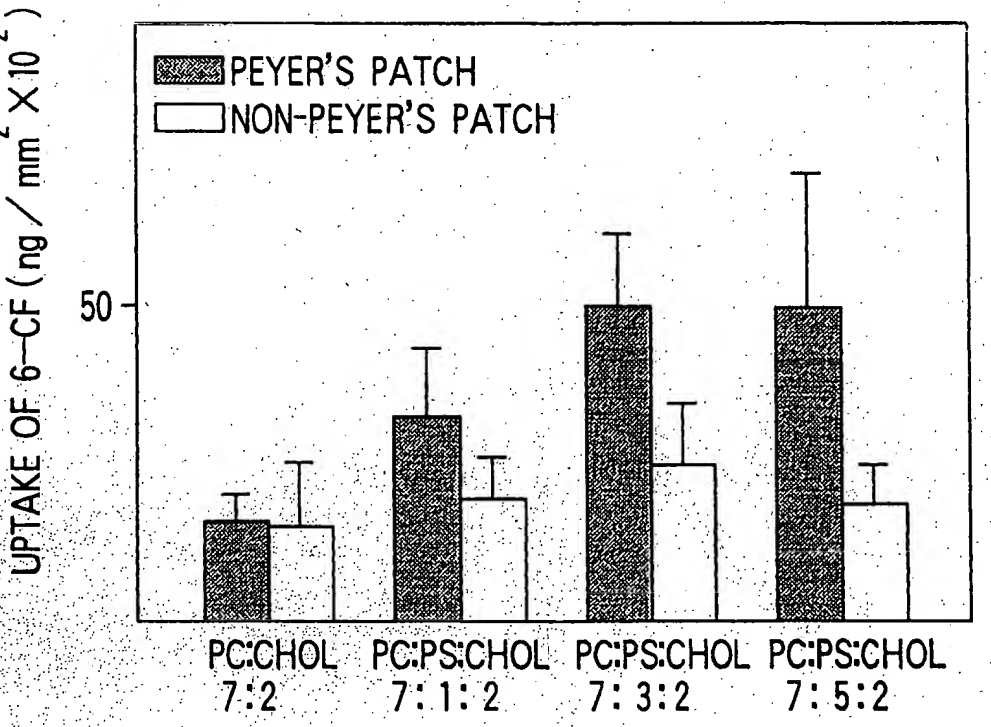

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Fig.3 EFFECT OF INCUBATION TIME ON THE UPTAKE OF PS-LIPOSOMES

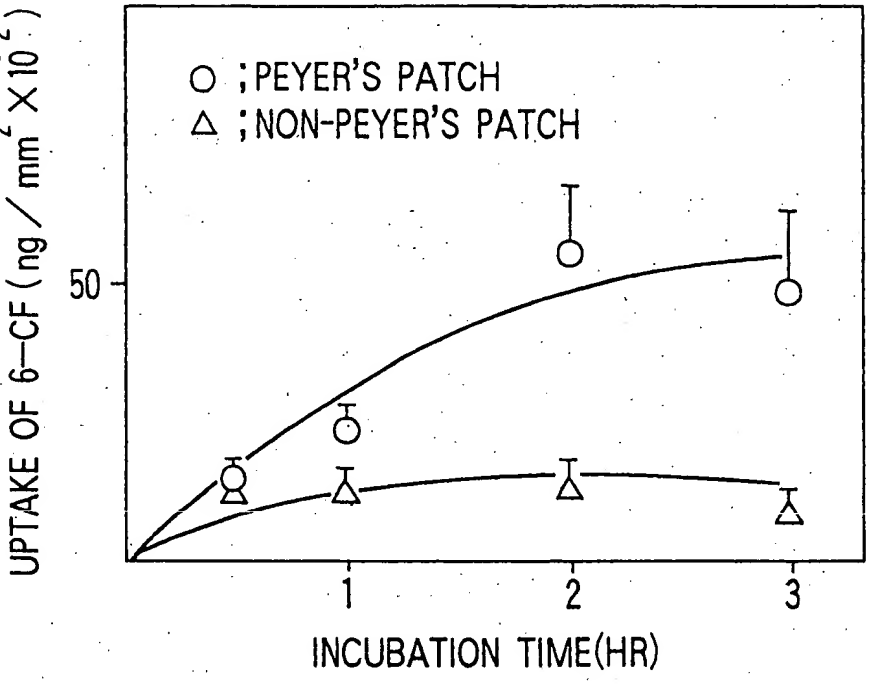

Fig.4 DIFFERENTIAL UPTAKE OF LIPOSOMES ON PEYER'S PATCHES

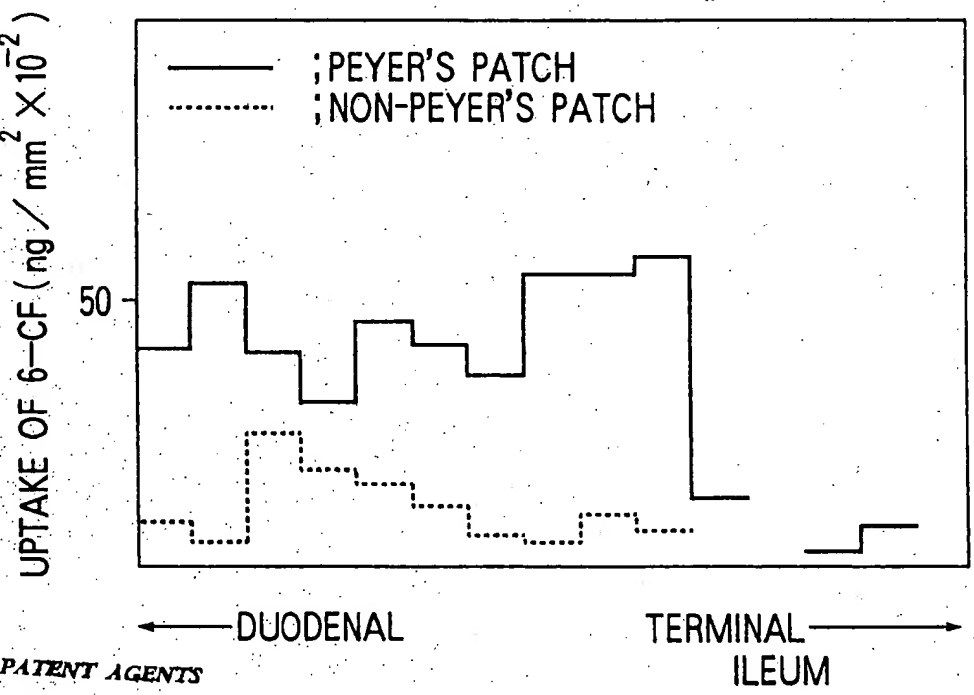

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Fig.5 EFFECT OF MANNAN MODIFYING ON THE UPTAKE OF PS-LIPOSOME

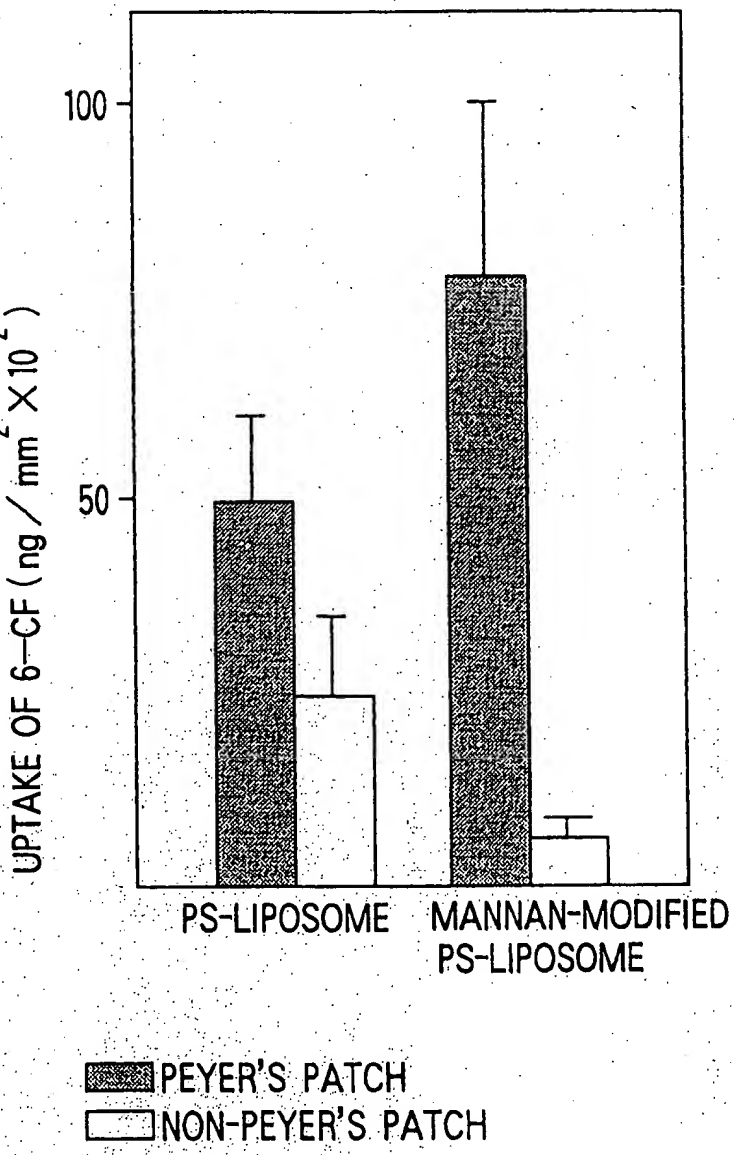

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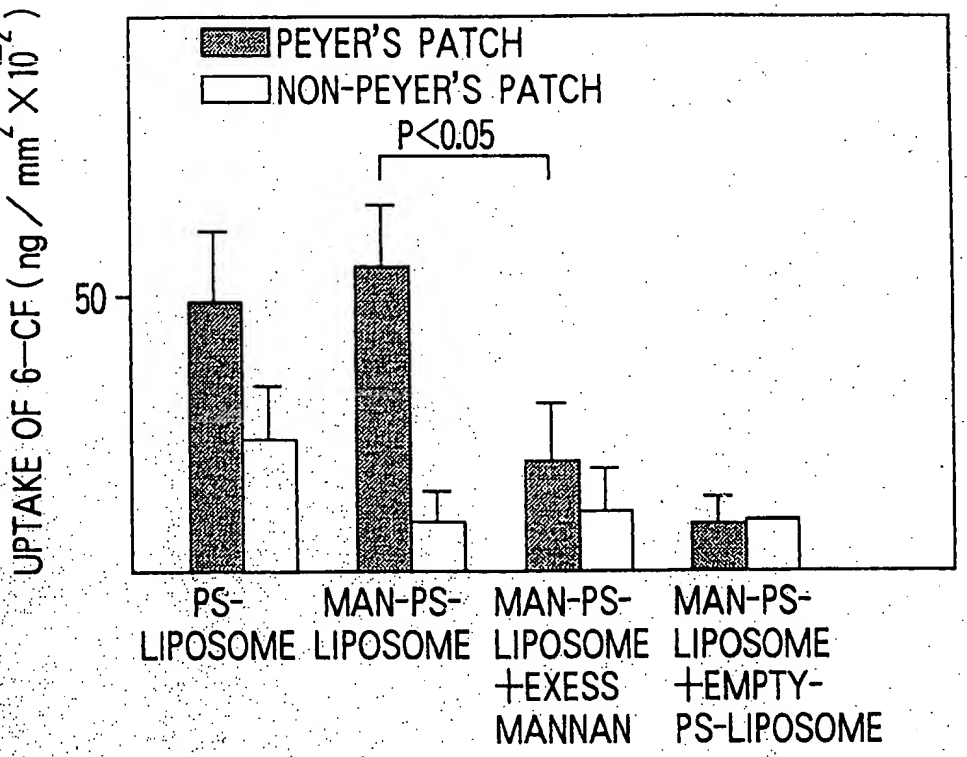
Fig.6 EFFECT OF MANNAN MODIFYING ON THE UPTAKE OF PS-LIPOSOME



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You are responsible for and warrant compliance with all applicable laws, rules and regulations, including but not limited to, customs laws, import and export laws and government regulations of any country to, from, through or over which your shipment may b You agree to furnish such information and complete and attach to this shipment such documents, or submit shipment data to FedEx, as necessary to comply with such laws, rules, and regulations. FedEx assumes no liability to You or any other person for any l expense due to Your failure to comply with this provision.

Letter of Instruction. If You do not complete all the documents required for carriage or if the documents submitted are not appropriate for the services or destination requested, You hereby instruct FedEx, where permitted by law to complete, correct or re documents for You at Your expense. However, FedEx is not obligated to do so. If a substitute form of air waybill is needed to complete delivery of Your shipment and FedEx completes that document, the terms of the FedEx Ship License and this document will to govern. FedEx is not liable to You or any other person for FedEx's actions on Your behalf under this provision.

Export Control. You authorize FedEx to act as forwarding agent for You for export and customs purposes. You hereby certify that all statements and information contained on all air waybills and SEDs relating to exportation are true and correct. You furth all Commercial Invoice information submitted via FedEx Ship is true and correct. You expressly authorize FedEx to forward all information of any nature regarding any shipment to any and all governmental or regulatory agencies which request or require such information. You acknowledge that civil and criminal penalties, including forfeiture and sale may be imposed for making false or fraudulent statements or for the violation of any United States laws on exportation, including but not limited to, 13 U.S.C. § 401; 18 U.S.C. § 1001; and 50 U.S.C. App. 2410. You acknowledge that this shipment is not being sent to any entity listed on the Department of Commerce's Denied Parties List 15 C.F.R. Part 764, Supp. 2, or the list of Special Designated Nationals as publi the Office of Foreign Assets Control of the U.S. Department of the Treasury.

Items Not Acceptable for Transportation. FedEx will not accept certain items for carriage, and other items may be accepted for carriage only to limited destinations or under restricted conditions. FedEx reserves the right to reject packages based upon th for reasons of safety or security. You may consult the FedEx Service Guide or Standard Conditions of Carriage for specific details.

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